Effect of reduced bronchial circulation on lung fluid flux after smoke inhalation in sheep

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1Departments of Anesthesiology and Physiology and Biophysics, University of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas 77555–0833; 2Department of Plastic and Reconstructive Surgery, Tokyo Women’s Medical College, Tokyo 180; and 3Department of Surgery, Yamaguchi University, Ube 755, Japan

Sakurai, Hiroyuki, Richard J ohnigan, Yuji Kikuchi, Mikihiro Harada, Lillian D. Traber, and Daniel L. Traber. Effect of reduced bronchial circulation on lung fluid flux after smoke inhalation in sheep. J. Appl. Physiol. 84(3): 980–986, 1998.—We determined the effect of reduced bronchial blood flow on lung fluid flux through changes in lung lymph flow, lung wet-weight to dry weight (wet/dry) ratio, and pulmonary microvascular reflection coefficient ($\sigma$). In the first of two surgical procedures, Merino ewes ($n = 21$) were surgically prepared for chronic study. Five to seven days later, in a second operation, the bronchial artery of the injection group ($n = 7$) was ligated, and $4 \text{ ml of } 70\%$ ethanol were injected into the bronchial artery to cause sclerosis of the airway circulation. In the ligation group ($n = 7$), only the bronchial artery was ligated. In the sham group ($n = 7$), the bronchial artery was surgically exposed but left intact without ligation or ethanol injection. One day after these operations the animals received a tracheotomy and $48 \text{ breaths of cotton smoke}$. The value of $\sigma$ was determined at two points: $24 \text{ h}$ before the second surgical procedure and $24 \text{ h}$ after smoke inhalation. Lung lymph flow, blood-gas parameters, and hemodynamic data were measured every $4 \text{ h}$ after injury. At the end of investigation, samples of lung were taken for determination of blood-free wet/dry ratio. In the sham group, injury induced a gradual increase in pulmonary vascular resistance and lung lymph flow, which was associated with deterioration of oxygenation. Reduced of the bronchial blood flow attenuated these pathophysiological changes, and the degree of this attenuation was greater in the injection group than in the ligation group. The value of $\sigma$ was significantly higher after smoke inhalation in the injection group compared with the sham group ($0.77 \pm 0.04$ vs. $0.61 \pm 0.03$, means $\pm SE$) at $24 \text{ h}$. The mean wet/dry ratio value of the injection group animals was $30\%$ less than that of the sham group. Our data show that the bronchial circulation contributes to edema formation in the lung occurring after acute lung injury with smoke inhalation.

lung lymphatic; reflection coefficient; pulmonary edema; bronchial artery; acute lung injury; burns; pulmonary microvasculature are delayed in onset, and the peak of increased microvascular permeability was observed around $24 \text{ h}$ after injury (16). In contrast, there is a marked increase in bronchial blood flow immediately after inhalation injury (1, 27). Because the increased bronchial blood flow enters into the pulmonary vasculature through various bronchopulmonary anastomoses (25), it has been suggested that the bronchial circulation plays a significant role in the spread of injury from the airway of the lung to the parenchyma (2, 11). Mechanical occlusion of the bronchial artery reduced lung edema formation after smoke inhalation in an anesthetized canine model (11) and in a conscious sheep model (2). However, this method could not totally abolish the pathophysiological changes that occurred in the pulmonary vasculature after smoke inhalation.

Because the sheep has a common bronchial branch arising from the bronchoesophageal artery, which supplies the lung, investigators have conducted a number of experimental studies of the bronchial circulation in this animal. Recent investigations, however, have shown that there are multiple systemic arteries to the lung in sheep, as in other species (8, 9, 19, 20). Baile et al. (4) have reported that mechanical obstruction of the bronchial artery may lead to opening of the collateral circulation, although the source of the collateral blood flow was not clearly defined. Consequently, they concluded that sclerosing the airway microvasculature with ethanol is a more effective procedure for abolishing the bronchial circulation (4). The purpose of the present study was to test whether ablation of the bronchial circulation could attenuate the increased pulmonary transvascular fluid flux after smoke inhalation.

METHODS

Animal care and use. Animals were cared for in the Ovine Intensive Care Unit at our institution, which is approved by the American Association of Laboratory Animal Care. The experimental procedures were approved by the Animal Care and Use Committee of The University of Texas Medical Branch. The National Institutes of Health and American Physiological Society guidelines for animal care were strictly followed. Animals were studied in the awake state with free access to food and water.

Surgical preparation. Twenty-one female range-bred adult Merino sheep (25–40 kg) were surgically prepared for study. All animals were endotracheally intubated and ventilated during the surgery under halothane anesthesia. Arterial and venous catheters (16 gauge, 24 in., Intracath, Becton Dickinson, Sandy, UT) were placed in the descending aorta and
inferior vena cava via the femoral artery and vein, respectively. A Swan-Ganz thermal dilution catheter (model 93A-131–7F, Edwards Critical-Care Division, Irvine, CA) was positioned in the right pulmonary artery via the right external jugular vein. The chest was opened at the fifth intercostal space in both sides, and an efferent lymphatic from the caudal mediastinal lymph node (CMN) was cannulated (Silastic medical-grade tubing, 0.025 in. ID, 0.047 in. OD, Dow Corning, Midland, MI) by a modification of the technique of Staub et al. (26). The systemic contribution was removed by ligating the tail of the CMN and catherization of the systemic diaphragmatic lymph vessels. Vascular occluders (In Vivo Metric System, Healdsburg, CA) were placed around each of the pulmonary veins as they entered the left atrium, as described by Isago et al. (16). A Silastic catheter was also positioned in the left atrium during the procedure to measure left atrial pressure directly. The sheep were given 5–7 days to recover from the surgical procedure with free access to food and water.

Ablation of bronchial circulation. After a 5- to 7-day recovery period, the animals were endotracheally intubated and ventilated during the surgery, which was performed under halothane anesthesia. In this operation, the animals were equally and randomly assigned to one of three groups. In the ablation group (n = 7), the left thorax was exposed through the fifth intercostal space, and the lungs were retracted, exposing the dorsal anatomy. During this procedure, a pleural adhesiotomy was performed. Then the bronchoesophageal artery was exposed, and 4 ml of 70% ethanol were injected into this artery through a lacrimal cannula (27 gauge, 30 mm, Nakamura-Ruikansenyosin-Kairyogusha, Handaya, Tokyo, Japan), after the ligation of the esophageal branch with 5–0 silk suture. In the ligation group (n = 7), the bronchoesophageal artery was isolated and tied off using 5–0 silk suture without ethanol injection. In the sham group (n = 7), the bronchoesophageal artery was exposed but left intact.

To substantiate the changes in bronchial blood flow, colored microspheres (Interactive Medical Technologies, West Los Angeles, CA) were injected. Immediately before and 24 h after the second operation, ~12 × 10^6 fluorescent colored microspheres (15.0 ± 0.1 µm) were injected into the left atrium of sheep; three major pulmonary veins are normally associated with the right lung and two with the left lung. Anatomically, these veins enter the left atrium separately, not as a common trunk, making it necessary to occlude each vein separately. We increased pulmonary arterial pressure by ~15–20 mmHg by infusing the pulmonary venous occluders with normal saline. In our experience, this volume was ~50% of the capacity of each occluder. If QL stabilized at this level, the occluders were further inflated in an attempt to obtain a filtration-independent state for CL, Qi, and Ci were measured every 30 min until Ci reached a minimal value at the highest PAP that the animals would tolerate. The pressure was kept stable for 120 min. The lymph protein levels became stable within 90 min after the pressures were raised, and the data were collected for analysis when the protein levels in the lymph had been stable at their lowest level. The reflection coefficient, α, was estimated from the minimal CI by using the formula: α = 1 – CI/QL.

Experimental protocol. The sheep were connected to pressure transducers and monitors for continuous monitoring of left atrial, central venous, right pulmonary arterial, and aortic pressures. The baseline data were determined 24 h after the second operation. Only the baseline data of α were determined 24 h before the second operation, since this measurement affects most hemodynamic and lung lymph data, as previously reported (16). After all baseline measurements were completed, all animals received 48 breaths of cotton smoke, as described above. Immediately after insufflation, anesthesia was discontinued and the animals were allowed to awaken but were ventilated mechanically with a Servo Ventilator 900C (Siemens-Elema, Solna, Sweden) throughout the next 24-h experimental period. Ventilation was performed with a positive end-expiratory pressure of 5 cmH2O and a tidal volume of 15 ml/kg. The inspiratory O2 concentration was adjusted to maintain the arterial O2 saturation above 90%. The respiratory rate was set to maintain normocapnia. Fluid resuscitation during the experiment was performed with Ringer lactate solution (3 ml·kg^-1·h^-1).

When all measurements were completed, the animals were killed by an infusion of ketamine followed by a saturated potassium chloride solution. Immediately thereafter, a necropsy was performed, and the right and left whole lungs were removed for measurement of blood-free wet weight-to-dry th.
weight (wet/dry) ratios, as described by Pearce et al. (23). About 400–500 mg of the bronchi (2–4 mm) tissue samples were obtained from left caudal lobe for microsphere measurements as described.

Analysis of data. All values are reported as means ± SE. Differences from baseline within the three groups were assessed post hoc by Dunnett’s test. Analysis of differences between groups was assessed by Scheffé’s test. Statistical significance was accepted at P < 0.05.

RESULTS

The arterial carboxyhemoglobin levels just after smoke exposure were 74.0 ± 5.0% in the sham group, 80.6 ± 4.2% in the ligation group, and 76.2 ± 4.9% in the injection group. These values were not statistically different from one another, reflecting the consistency of the injury in each group.

The changes in blood flow to intraparenchymal bronchi (2–4 mm) before and after the second operation are shown in Fig. 1. In the sham group, blood flow was not significantly changed. The reduction of airway blood flow in the ligation and injection groups was 32.1 ± 12.0% and 86.3 ± 7.2%, respectively. The intrapulmonary airway blood flow in the injection group was significantly less than those in both the sham and ligation groups.

The changes in cardiopulmonary hemodynamics are shown in Table 1. Mean arterial pressure and filling pressures were maintained at baseline level, although there was a statistically insignificant trend for cardiac index to fall in all groups. The sham group showed a significant increase in PAP at 24 h after smoke inhalation, whereas in the ligation and injection groups the increase in PAP was mild and was not statistically different from the baseline value. There were no significant differences between groups at any time.

The changes in intrapulmonary airway blood flow are shown in Table 1. The injection group was significantly less than in the sham group at 24 h after smoke insufflation.

Table 1. Cardiopulmonary hemodynamics

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>12 h</th>
<th>24 h</th>
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<tbody>
<tr>
<td>CI, l·min⁻¹·m⁻²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>6.17 ± 0.21</td>
<td>5.56 ± 0.34</td>
<td>5.01 ± 0.57</td>
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<tr>
<td>Ligation</td>
<td>5.41 ± 0.15</td>
<td>4.86 ± 0.21</td>
<td>4.93 ± 0.19</td>
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<tr>
<td>Injection</td>
<td>6.19 ± 0.45</td>
<td>6.16 ± 0.53</td>
<td>6.04 ± 0.48</td>
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<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>96 ± 3</td>
<td>95 ± 3</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Ligation</td>
<td>95 ± 3</td>
<td>96 ± 3</td>
<td>93 ± 4</td>
</tr>
<tr>
<td>Injection</td>
<td>105 ± 5</td>
<td>103 ± 5</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>23 ± 2</td>
<td>28 ± 2</td>
<td>33 ± 2*</td>
</tr>
<tr>
<td>Ligation</td>
<td>23 ± 1</td>
<td>25 ± 2</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Injection</td>
<td>25 ± 1</td>
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<td>29 ± 2</td>
</tr>
<tr>
<td>CVP, mmHg</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Ligation</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Injection</td>
<td>9 ± 1</td>
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<tr>
<td>LAP, mmHg</td>
<td></td>
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<tr>
<td>Sham</td>
<td>8 ± 1</td>
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<tr>
<td>Ligation</td>
<td>8 ± 1</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
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<tr>
<td>Injection</td>
<td>10 ± 1</td>
<td>14 ± 2</td>
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</table>

Fig. 1. Changes in intrapulmonary airway blood in 3 groups. Open bars, before 2nd operation; solid bars, 24 h after 2nd operation. Values are mean ± SE. *Significant difference (P < 0.05) vs. baseline. †Significant difference (P < 0.05) vs. sham group. #Significant difference (P < 0.05) vs. ligation group.

Fig. 2. Systemic vascular resistance index (SVRI; A) and pulmonary vascular resistance index (PVRI; B) in sham (intact bronchial artery; n = 7), ligation (ligated bronchoesophageal artery; n = 7), and injection (ethanol injection into bronchial artery; n = 7) groups. *Significant difference (P < 0.05) vs. baseline. †Significant difference (P < 0.05) vs. sham group.

the injection group was significantly less than in the sham group at 24 h after smoke insufflation.

Figure 3 depicts oxygenation after smoke inhalation injury. Mechanical ventilation with adjusted fractional concentration of inspired O2 (FIO2) allowed each group to maintain arterial P O2 (PaO2) above baseline levels.
Progressively deteriorated oxygenation was observed in the sham group, represented by the decreased PaO2/FIO2 ratio. This deterioration was significantly attenuated in the injection group during the 24-h period. In the ligation group, the fall of PaO2/FIO2 ratio was delayed, but 24 h after smoke inhalation, this value had significantly dropped from the baseline value.

Q˙L increased to nearly four times the baseline value by 24 h after insult in the sham group (Fig. 4). This increase was significantly reduced in the ligation and injection groups. The increase in Q˙L in the sham group was associated with a significant increase in the LPC (Fig. 5). In the ligation group, this increase was less but not significantly different from the increase seen in the sham group. In the injection group, however, this increase was further reduced, and the protein clearance at 24 h was significantly lower than in the sham group.

The reflection coefficient, $s$, decreased significantly in the sham group (Fig. 6). In the injection group, the fall in $s$ was small and was not statistically different from the baseline value. The value of $s$ of the injection group was statistically higher than that of the sham group 24 h after smoke insufflation. The ligation group showed an intermediate decline in $s$.

Figure 7 shows blood-free wet/dry ratios of both right and left lungs. The control represents normal value in our laboratory from six healthy animals without any injury. The sham group showed significant increases when these values were compared with corresponding lungs from control animals. In the ligation and injection groups, these increases were less remarkable and statistically insignificant from the control values. The left lung wet/dry ratios in both the ligation and injection groups showed significant differences vs. the corresponding value of the sham group.
Control value. †Significant difference (P < 0.05) vs. sham group.

DISCUSSION

The findings of the present study confirm our hypothesis that ablation of the bronchial circulation dramatically attenuates the pathophysiological changes that occur after smoke inhalation. In our previous report (2), we suggested that the bronchial circulation might contribute to the pathogenesis after smoke inhalation, but this suggestion remained unsubstantiated, since the mechanical occlusion of the bronchial artery affected only a few parameters and the magnitude of the attenuation was limited.

The anatomy of the bronchial circulation of the ovine lung has been carefully documented (8, 9, 19, 20). Charan et al. (8) showed that sheep have multiple sources of systemic arterial blood flow to the lung and that these arteries anastomose with each other as well as with the pulmonary circulation. Ashley et al. (3) investigated the effect of transient occlusion of the bronchoesophageal artery and demonstrated 70–90% reduction of the intrapulmonary airway blood flow at 5 min after the occlusion. In the present study, the reduction of the systemic blood flow into the same-size airway at 24 h after ligation of the bronchoesophageal artery was merely 32%. This finding suggested that collateral circulation to the intrapulmonary airway could be easily established within a day.

Baile et al. (4) used radioactive microspheres and reported that ethanol injection into the bronchoesophageal artery reduced systemic arterial blood flow into the sheep lung by ~75%, whereas ligation of the bronchial artery resulted in a ~50% reduction. These values cannot be purely interpreted as a reduction of the intrapulmonary airway blood flow, since the systemic arterial blood supply to the lung is delivered not only to the airway but also to the adventitia of large vessels and structures of the lungs (10). However, ethanol injection is certainly a more reliable method than mechanical occlusion of the bronchoesophageal artery, and in the present study regional blood flow into the intrapulmonary airway substantiated the ablated bronchial circulation in the injection group.

Besides these anatomic considerations about airway blood flow, the findings in the ligation group strongly suggested that the collateral airway blood flow might be responsible for the incomplete block of the pathophysiological changes after smoke inhalation. In the ligation group, Q˙L and LPC were significantly increased 24 h after injury, and oxygenation was notably decreased. With a more complete elimination of the bronchial circulation, the injection group showed a greater ability to attenuate the pathophysiological effects associated with smoke inhalation injury. There were no significant changes in Q˙L, LPC, oxygenation, or PVR during the whole experimental period. In addition, σ at 24 h after injury was not significantly different from the baseline value. In contrast, all these variables showed statistical differences when compared with the sham group at 24 h after injury.

The mechanism by which the bronchial circulation contributes to the pathogenesis in lung parenchyma has not been clearly defined. The bronchial circulation itself might be a source of the lung edema. Hales et al. (11) used an acute canine model with intravenous injection of dye and histologically demonstrated increased permeability in the bronchial vascular bed after synthetic smoke insufflation. Because our methods of analyzing lung fluid flux do not allow us to detect the proportional contribution of the bronchial circulation, increased Q˙L, LPC, and σ might originate from the bronchial vasculature. However, it is noteworthy that no parameters measured after the second operative procedure showed significant differences between groups. These findings suggested that the contribution of bronchial circulation to Q˙L or other parameters is not so significant, at least before smoke inhalation. We have previously demonstrated a three- to fivefold increase in bronchial arterial blood flow after smoke inhalation (1, 27); however, this flow was still <2% of cardiac output. Considering that total blood flow perfusing pulmonary vasculature is equal to cardiac output, the contribution of the bronchial circulation to the total vascular bed in the lung (pulmonary and bronchial circulations) is very small. Because increased Q˙L is a manifestation of fluid that traversed the total vascular bed in the lung, it is hardly expected that a major part of the increased lymph flow originated from bronchial vascular bed.
In our conscious animal model, the pulmonary permeability changes after smoke inhalation are considerably delayed (2, 15, 17), and the peak of increased pulmonary microvascular permeability is observed around 24 h after injury (16). In contrast, increased permeability in bronchial vessels occurs more rapidly, as demonstrated by Hales et al. (11). Barrow et al. (6) also demonstrated a more rapid permeability change in the extrapulmonary airways than that in the lungs. The time course differences between the pulmonary and the bronchial circulation, and the anatomic observation that most of intrapulmonary airway blood flow drained into the pulmonary circulation (5, 9), led us to consider that increased bronchial blood flow played a significant role in the spread of injury from the airway to the pulmonary vasculature.

In the sham group, there was a gradual increase in PVR associated with a significant rise in pulmonary vascular pressure and a mild drop in cardiac output. We have previously reported that pulmonary venous resistance increased proportionally more than pulmonary arterial resistance after smoke inhalation (14). In the present study, increased PVR was significantly attenuated by ablation of the bronchial circulation. There are several putative mediators responsible for the increased PVR associated with smoke inhalation injury. Quinn et al. (24) reported that leukotriene D₄ is found in lung lymph and pulmonary edema fluid after synthetic smoke exposure in sheep and that pretreatment with a leukotriene-receptor antagonist attenuated the PVR increase and decrease in cardiac output. Furthermore, Noonan et al. (21, 22) showed that leukotriene D₄ infusion in sheep caused increases in PAP and PVR, primarily by inducing thromboxane formation. Additionally, we reported that PAP, PVR, and Qₑ were significantly attenuated in chronically prepared sheep that had been made leukopenic with intraarterial infusions of nitrogen mustard (7). These findings suggest that leukotriene, thromboxane, and some mediators released from leukocytes might be associated with the increase in PVR after smoke inhalation. The most interesting point, however, is that these vascular changes occur specifically in the pulmonary and not in the systemic vasculature, since SVR is essentially unchanged after smoke inhalation.

The present study showed that the bronchial circulation plays a significant role in lung edema formation after smoke inhalation. On the other hand, there is increasing evidence that the bronchial vasculature is also involved in edema clearance. Recently, Fukue et al. (10), using in situ perfused sheep lung, reported that up to 14% of hydrostatic edema might be reabsorbed by the bronchial circulation. Even though our present data do not support the role of bronchial circulation in the clearance of edema, under certain conditions the bronchial circulation can take on the edema-absorbing function. Furthermore, this circulation might be important for regeneration of airway mucosa during the proliferative or reparative phase after smoke inhalation (18). Further investigations are required to elucidate the longitudinal effect of ablated bronchial circulation.

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